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AP - JP19950147672 19950614; WO1996JP01579 19960611; WO1996JP01579 19960611; JP19970502907 19960611; [Based on WO9700020]

PR - JP19950147672 19950614

TI - Oral composition for animals contg. heat resistant enzyme - pref. cellulase, xylanase, protease, lipase or amylase, used in feeds, foods and medicines, for humans, livestock, etc.

IW - ORAL COMPOSITION ANIMAL CONTAIN HEAT RESISTANCE ENZYME PREFER CELLULASE XYLANASE PROTEASE LIPASE AMYLASE FEED FOOD MEDICINE HUMAN LIVESTOCK

PA - (SHOW) SHOWA DENKO KK
- (NOVO) NOVO-NORDISK AS

PN - JP2001057852 A 20010306 DW200118 A23K1/165 007pp
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AB - WO9700020 Oral composition for animals contg. heat resistant enzyme, which when heated to 80 deg. C for 20 seconds retains more than 60% activity. Also claimed is a method for producing the composition.
- The enzyme is pref. cellulase, xylanase, protease, lipase or amylase derived from Bacillus spp.
- USE - The composition is used in feeds, foods and medicines(claimed), for humans, livestock, pets, fish and insects.
- ADVANTAGE - The composition is stably stored at room temp. for extended periods, and is simple and economical to produce. It is easily digested.
- (Dwg.0/0)

PATENT ABSTRACTS OF JAPAN

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(71)Applicant : SHOWA DENKO KK
(72)Inventor : KAMAIKE MOTOAKI
SATO HAJIME
ITO SHINOBU

(54) HEAT-RESISTANT ENZYME-CONTAINING FEED COMPOSITION

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a feed composition fully retaining enzyme activity even after undergoing a process with a heating-type equipment, fortified in its functionality and nutritive value and excellent in storability and digestibility in the body of livestock by including a heat-resistant enzyme having a specified residual activity of at least a specific value.
SOLUTION: This feed composition is obtained by including at least one heat-resistant enzyme having a residual activity of ≥ 60% when heated at 80°C for 20s and selected from cellulase, xylanase, protease, lipase and amylase. It is preferable that the heat-resistant enzyme is an enzyme obtainable from e.g. Bacillus sp.SD401(FERM P-11527), SD402 (FERM BP-3431), SD403(FERM P-11647), SD771(FERM P-11455), SD772(FERM P-11456), SD902(FERM BP-4508), NKS-21(FERM BP-3911), and also the feed composition is produced by addition of the heat-resistant enzyme followed by heating with a heating-type equipment at ≥ 70°C.

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CLAIMS

[Claim(s)]
[Claim 1] The constituent for feed containing the thermostable enzyme whose residual activity when heating for 20 seconds at 80 degrees C is 60% or more.
[Claim 2] The constituent for feed according to claim 1 with which a thermostable enzyme is chosen from a cellulase, xylanase, a protease, lipase, or an amylase and which is a kind at least.
[Claim 3] The constituent for feed according to claim 1 or 2 whose thermostable enzyme is an enzyme obtained from the culture of the Bacillus bacteria.
[Claim 4] A thermostable enzyme Bacillus sp.SD401 (FERM P-10527), SD402 (FERM BP-3431), SD403 (FERMP-11647), SD771 (FERM P-11455), SD772 (FERM P-11456), SD902 (FERM BP-4508), NKS-21 (FERM BP-93-1), Bacillus licheniformis Constituent for feed according to claim 1 or 2 which is the enzyme which can be obtained from SD516 (FERM P-10427).
[Claim 5] The manufacture method of the claim 1 including the process heated at 70 degrees C or more by the heated type device after adding a thermostable enzyme, or the constituent for feed given in 4.
[Claim 6] The manufacture method of the constituent for feed according to claim 5 that a heated type device is a pelleter machine, an extruder, an expander, or a dryer.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] this invention relates to producing a constituent for feed which is characterized by for an enzyme existing in the constituent for feed stably, and having activity by blending the enzyme which has thermal resistance as a constituent for feed, and using it.
[0002]
[Description of the Prior Art] The enzyme is used in the form which is one of the feed additives, and is added and mixed in much feed. As for one of the purpose of use of the, generally, it is main to connect with to promote the digestion of feed which carried out ingestion in the livestock body, and promoting decomposition of the fiber in a forage crop as other purposes at the time of silage processing of feed to good lactic acid fermentation. About these, it doubles with each purpose and the enzyme suitable for the condition is found out. Moreover, the method of mixing those addition methods in the feed the time of a feed salary or beforehand, and feeding to livestock and the method of adding an enzyme agent with lactic acid bacteria in silage processing are taken.
[0003] However, generally, an enzyme deactivates and it is easy to disassemble it in an elevated-temperature field. Therefore, if it passes through a manufacturing process which includes a high-temperature-processing process like mixed feed at the time of manufacture, it is very difficult to deactivate promptly and to make the effect fully maintain. Especially in the latest feed manufacture, heated type granulating machines which process a raw material at the elevated temperature of 70 degrees C or more from the reasons of a moldability, handling nature, etc., such as a pellet mill and an extruder, are used. Although there was no problem about stability when it was the feed manufacture conditions processed at the slow temperature of 65 degrees C or less, in the manufacturing process of the feed by the elevated-temperature briquetting machine processed above 70 degrees C like manufacture of the pellets which are excellent in moldability and a configuration, there was a problem that most of these matter will be decomposed. Therefore, development of the technology which blends an enzyme stably has been demanded.
[0004] In order to stabilize an enzyme, there is technology made into coating or an enzyme premix. For example, there are JP,6-504911,A and JP,63-157938,A. However, the coating enzyme is disadvantageous in cost and behind in spread. moreover, when the coated enzyme agent is destroyed at the pulverization process of the feed manufacturing process preceding paragraph, in order that heat or moisture may contact a direct enzyme, possibility that it will be alike among the manufacturing process of feed, circulation process, and a retention period, deactivation will arise, and the effect will decrease sharply is high [the point common to coating technology] In an enzyme premix, it cannot be said that the process of premix-izing doubled with the enzyme is required, and the manufacturing process using the heated type device is enough also as the stability.
[0005] Moreover, depending on feed, there is what has high moisture content like the feed for fishery, or the feed for silkworms. This has the feature at which the water-soluble nutrition component in feed is eluted promptly, and the firmness of feed is fully maintained. However, although an enzyme generally excels [stability] in shelf life highly in a dryness article, possibility that stability will be low, deactivation will progress and the effect will decrease sharply is high in the field where moisture content is high. On the manufacturing process conditions of the feed by the heating-at-high-temperature type briquetting machine processed above 70 degrees C like

manufacture of the pellets which are excellent in especially moldability and a configuration, this deactivation is accelerated further. Therefore, application of the enzyme in feed with the high content of moisture is made very difficult.
[0006] Moreover, the present condition requires the feed which is equal to a mothball from facilitation of quality control, and a transportation cost mitigation side, although mixed feed is used for the short period of time after manufacture from the point of quality maintenance. For this reason, the cure for the inside of a retention period also maintaining the enzyme activity immediately after manufacture is needed.
[0007]
[Problem(s) to be Solved by the Invention] In case an enzyme is used for this invention as one component of the constituent for feed, it is for the manufacturing process of feed which used the heating-at-high-temperature type device to also offer the stable constituent for feed also to the preservation of feed over a long period of time, without this enzyme deactivating.
[0008]
[Means for Solving the Problem] The manufacturing process of the feed with which this invention persons used the heating-at-high-temperature type device is not decomposed, either. And it gropes for the enzyme which has the enzyme activity which brings about an effective effect to the extensive animals bred. As a result of inquiring, when using the enzyme which has thermal resistance, it finds out that sufficient feed-effect which the stability can be raised by leaps and bounds, and originates in the enzyme activity in extensive animal species is demonstrated, and came to complete this invention.
[0009] That is, this invention offers the following.
1) The constituent for feed containing the thermostable enzyme whose residual activity when heating for 20 seconds at 80 degree C is 60% or more.
2) The constituent for feed given in the above 1 with which a thermostable enzyme is chosen from a cellulase, xylanase, a protease, lipase, or an amylase and which is a kind at least.
[0010] 3) The above 1 whose thermostable enzyme is an enzyme obtained from the culture of the Bacillus bacteria, or the constituent for feed given in 2.
A thermostable enzyme 4) Bacillus sp.SD401 (FERM P-10527), SD402 (FERM BP-3431), SD403 (FERM P-11647), SD771 (FERM P-11455), SD772 (FERM P-11456), SD902 (FERM BP-4508), NKS-21 (FERM BP-93-1), Bacillus licheniformis The above 1 which is the enzyme which can be obtained from SD516 (FERM P-10427), or constituent for feed given in 2.
[0011] 5) The manufacture method of the above 1 including the process heated at 70 degrees C or more by the heated type device after adding a thermostable enzyme, or the constituent for feed given in 4.
6) The manufacture method of the constituent for feed given in the above 5 whose heated type device is a pelleter machine, an extruder, an expander, or a dryer.
[0012] Hereafter, this invention is explained still in detail.
(Enzyme) The enzyme added by the constituent for feed of this invention Into the cooker portion which performs gas conditioning and cooking in the conditioner portion and extruder which perform steam heating in a part for a heating unit, for example, the pellet mill, of the heating-at-high-temperature type briquetting machine heated at 80 degrees C or more What is necessary is just a kind or two sorts or more of enzymes with which the residual activity of the enzyme in the feed made to pile up for 20 seconds at least is desirably chosen from the enzymes which have thermal resistance which has the property which shows 90% or more of residual activity more desirably 80% or more at least 60%. And as for these enzymes, it is desirable to have a property which promotes the digestion in the digestive organs in a rearing animal, the property in which the living body function of a rearing animal is improved, or the property to change the component in the feed in which a rearing animal carries out ingestion to a gestalt useful for the animal. For example, the enzyme obtained from the culture medium of bacillus sp.SD771 (FERM P-11455) or bacillus sp.SD772 (FERM P-11456), Especially An amylase (JP,4-23983,A, JP,4-58885,A), The enzyme obtained from the culture medium of bacillus sp.SD902 (FERM BP-4508), The variant by the protein engineering of the protease obtained especially from xylanase (JP,6-261750,A) and bacillus sp.NKS-21 (FERM BP-93-1), For example, the alanine variant of the 12th serine (JP,5-91876,A), Bacillus sp.SD401 (FERM

P-10527), bacillus sp.SD402 (FERM BP-3431), Bacillus sp.SD403 (FERM P-11647), Especially although there is an enzyme group (JP,2-215381,A, JP,4-79882,A, JP,4-104788,A, JP,5-209385,A) obtained from the culture medium of bacillus sp.SD516 (FERM P-10427), it is not limited to this.
[0013] As the manufacture method of an enzyme, when using a microorganism, as the cultivation method, the usual bacterial culture methods, such as liquid culture and a solid culture, can be used. If it carries out from the standpoint of economical efficiency, the method of carrying out liquid culture aerobically will be desirable, and the aeration spinner-culture method and shaking cultivation under aerobic conditions will be mentioned as the example. Although arbitrary culture-medium components can be used if the strain to be used may propagate as the culture medium For example, that what is necessary is just a thing containing the carbon compound or this which can be assimilated as a carbon source, if it is manufacture of xylanase independent in saccharides, such as starch hydrolyzates, such as various raw materials containing a xylan or xylan system polysaccharides, such as various xylans, wheat wheat bran, a pulping waste liquor, grain ******, or straw, or a glucose, starch, or liquefaction starch, and molasses, -- or it can combine and use
[0014] moreover -- for example, the thing containing the nitride or this which can be assimilated as a nitrogen source -- it is -- ****ing -- as an organic-nitrogen inclusion -- various amino acid, corn steep liquor, a malt extract, a peptone, a soybean meal, defatted-soybean powder, etc. -- moreover, independent [in ammonium salts, such as an ammonium chloride and an ammonium sulfate, etc.] as an inorganic-nitrogen compound -- or it can combine and use In addition, salts, such as the thing containing various kinds of organic substance, inorganic substance, or this required for growth and enzyme production of a bacillus, for example, phosphate, magnesium salt, a calcium salt, and manganese salt, vitamins, a yeast extract, etc. can also be added suitably.
[0015] Moreover, when using vegetation, an enzyme can be obtained in part by the extraction from an organ and extraction from the whole plant body, the culture medium obtained by the vegetable cultivation methods, such as liquid culture and a solid culture, or a cultured cell. if it is an animal -- the -- the target enzyme can be obtained in part from the culture medium of extraction from internal organs and an organ, or animal cell cultivation The culture medium used for cultivation can use arbitrary culture media, if it seems that the cultured cell increases and the target enzyme is produced. Moreover, about these extraction, generally the buffer solution is added under low temperature like ice-cooling, and the filtrate which carried out **(ing) or filter filtration after homogenizing and carrying out centrifugal separation of the organization is used as a rough refining enzyme solution. Furthermore, operation of centrifugal separation, filtration, sedimentation, concentration, dryness, etc. can be chosen suitably, and can be refined to this, and the refining enzyme solution can also be used.
[0016] The enzyme as a constituent for feed in this invention can take the medication dosage forms of all the types made from the object for animal breeding in order to medicate the animal inside of the body with a nutrition or useful chemicals, and has the feed for livestock, the premix agent for livestock, the vitamin compound for livestock, the drug for livestock, the feed for fish breeding, the premix agent for fish breeding, a vitamin compound for fish breeding, a drug for fish breeding, etc. as a typical example.
[0017] (Measuring method of enzyme activity) In the case of xylanase, about the existence of the xylan decomposition activity of the enzyme solution, it can investigate by the method as shown below as a shortcut method. That is, it suspends so that the xylan (SIGMA company make) of the auto wheat origin of marketing in the solution adjusted to arbitrary pH may become 1% of the weight of concentration, and after adding an agar so that it may become 2% of the weight, it heats to 90-100 degrees C, and an agar plate is produced. After diluting an enzyme solution suitably, or condensing it and carrying out a spot to an agar plate, it is kept warm at arbitrary temperature. If an agar plate is observed after 24-hour progress and a clear zone is accepted around a spot, it is possible to judge with xylan decomposition activity having. If the agar plate which added the substrate suitable about other enzymes is produced, the decomposition activity can be similarly judged by the existence of the clear zone of the spot circumference.
[0018] the xylanase activity-measurement method -- as a substrate -- the auto spelling in 50mM sodium acetate (pH 5.0) -- an ibis, although 39 degrees C and the amount of enzymes which generates 1micro mol of xylose in 1 minute by pH 3.5 were made into one unit (U) using the silane

(sigma company) There is much what has the feature in the property in an elevated-temperature field in the enzyme used by this invention. Depending on an enzyme, the birch xylan (hippo origin xylan) in 0.1M phosphate buffer solution (pH 7.0) is used as a substrate according to JP,6-261750,A. After making it react by 50 degrees C and pH 7.0, the generated reducing sugar were measured by 3 and 5-dinitro salicylic acid (DNS) which is a well-known method, and the amount of enzymes which generates 1micro mol of xylose in 1 minute was made into one unit (SU).

[0019] (Constituent for feed) The constituent for feed of this invention is used as the feed matter for livestock, domestic fowls and pet animals, such as fishery animals, such as land animals, such as a pig, a hen, a cow, a horse, a dog, a cat, and a silkworm, a salmon, a yellowtail, a mass, a carp, a sweet fish, a shrimp, and an eel, fishes, crustacean, and Insecta. Although, as for the constituent for feed of this invention, the effect shows up when a heated type device is used by the manufacturing process, when a heating pressurization type briquetting machine is especially used also in it, the effect is demonstrated more notably. Producing feed by the heating pressurization type briquetting machine is the effective means which may be made very cheaply rather than it adds alpha starch, when promoting the stability of feed, since the starch in feedstuff can be pregelatinized. However, feedstuff needs to be simultaneously pressurized by pregelatinization of starch at the elevated temperature of 70 degrees C or more. Depending on 70 degrees C or the case, since it is stable also under the elevated temperature beyond it, heatproof type enzymes become very useful [adding to feedstuff with the need of making alpha starch generating].

[0020] Moreover, maintaining the moisture content of the feed which is the constituent for feed of this invention to 30% or less prevents generating and decomposition of mold, and since feed is saved over a long period of time, it has an important meaning. For this reason, although it is the means which may be made the simplest and cheaply when carrying out elevated-temperature ventilation dryness of the feed by which heating pressurization molding was carried out in the manufacturing process considers the productive efficiency of feed Since heatproof type enzymes are sufficient dryness conditions to check hardly decomposing by experiment and for this condition make the moisture of usual feed 30% or less even if it dries under an elevated temperature with a ventilation temperature of 70 degrees C for 1 hour, In order to produce the feed for such a low water flow efficiently, addition of heatproof type enzymes becomes very useful.

[0021] As a heated type device of this invention, as a model of briquetting machine, a pellet mill, The possible molding equipment of heating used in order [, such as a pelleter machine, an extruder, and a knockout briquetting machine] to pelletize feed generally, There is a dryer of a cast etc. And fluid bed granulation coating equipment, a centrifugal flow type coating granulator, A centrifugal flow type granulating machine, a spray dry formula granulating machine, a rotating-disk formula fluidized bed granulator, a centrifugal rolling formula granulator, a suspension fluid type granulating machine, a revolution fluid type granulating machine, etc. can be set and used for the use timely.

[0022] The constituent for feed of this invention can use the matter equivalent to what doubled with the animal species which feeds and has been used conventionally. For example, wheat, grass, cereals, legumes, ******, an agricultural production workpiece, a zootechnics workpiece, a food-processing article, agricultural production waste, a livestock waste, food waste, oil, amino acid, vitamins, various salts, etc. are mentioned. The effect by the enzyme can also be further heightened by using the matter containing many components used as the substrate of an enzyme. The constituent for feed made into the purpose is obtained using the equipment of point ** that what is necessary is just to mix the enzyme in this invention with other constituents for feed of point ** in the configuration of fine particles and a liquid at the time of the manufacture. An addition can be doubled and set as the raw material and object animal which constitute feed.

[0023] The enzyme or enzyme solution used by this invention can be manufactured using the culture-medium component doubled with the strain to be used and the enzyme made into the purpose, and when using it with an enzyme solution, processing can be advanced having used more efficiently [direction] in the state where it condensed suitably.

[0024]

[Example] Hereafter, an example and the example of comparison explain this invention still in detail. However, this invention is not limited by the following examples and the example of comparison.

[0025] [Example 1] 3.0% peptone, 1.0% water-soluble-chlorophyll-derivatives, and 1.0%DE-50 or

1.0% yeast-extract, 1.0% wheat wheat bran, 0.5% potassium-phosphate, 0.05% magnesium sulfate, and liquid-medium which consists of sodium carbonate 0.5% 2L was put into the jar fermenter of 5L **, and it sterilized for 20 minutes at 121 degrees C. bacillus sp. cultivated by the same culture medium as this -- the biomass of SD771 (FERM P-11455) and SD772 (FERM P-11456) was inoculated respectively, and the aeration spinner culture was aerobically performed at 55 degrees C for 48 hours After cultivation, 6000rpm centrifugal separation of each culture medium was carried out, and the biomass was removed. Next, the supernatant obtained by the above-mentioned method was respectively condensed about 10 times by ultrafiltration, the calcium chloride was added and the enzyme solution was respectively obtained so that it might be set to final concentration 2mM to this.
[0026] A [example 2] 1.0% birch xylan, 0.1% yeast extract, 1.0% poly peptone, 0.5% potassium phosphate, 0.05% magnesium sulfate, 0.002% iron sulfate, and liquid-medium (pH 7.0) 2L that consists of a sodium chloride 0.05% were put into the jar fermenter of 5L **, and it sterilized for 20 minutes at 121 degrees C. The biomass of bacillus sp.SD902 (FERM BP-4508) cultivated by the same culture medium as this was inoculated, and the aeration spinner culture was aerobically performed at 55 degrees C for 48 hours. After cultivation, 6000rpm centrifugal separation of the culture medium was carried out, and the biomass was removed. Next, the supernatant obtained by the above-mentioned method was condensed by ultrafiltration, and the enzyme solution with which 200 SU/ml xylanase activity is accepted was obtained.
[0027] A [example 3] 2.0% soybean meal, 0.5% yeast extract, 0.5% sodium chloride, 0.1% potassium phosphate, 0.02% magnesium sulfate, 0.5% maltose, 0.1%CMC, and liquid-medium 2L that consists of a sodium carbonate 0.3% were put into the jar fermenter of 5L **, and it sterilized for 20 minutes at 121 degrees C. The biomass of bacillus sp.SD401 (FERM P-10527) cultivated by the same culture medium as this, SD402 (FERMBP-3431) and SD403 (FERM P-11647), and Bacillus licheniformis SD 516 (FERM P-10427) was inoculated respectively, and the aeration spinner culture was aerobically performed at 35 degrees C for 35 hours. After cultivation, 6000rpm centrifugal separation of each culture medium was carried out, and the biomass was removed. Next, the supernatant obtained by the above-mentioned method was respectively condensed by ultrafiltration, and the enzyme solution was obtained respectively. SD516 of the enrichment factor was about 10 times about 8 times and except it.
[0028] about 56% of [example 4] fish meals, and a salmon -- 6% of albinos, krill S.W.5%, and 5% of cuttlefish viscus 5% [of cuttlefish liver oil], 4% [of soybean lecithins], amino acid mix 0.5%, and corn-starch 5%, After considering as a total of 100% at magnesium 0.01% vitamin premix 2.8% gluten 5% mineral mix 5%, the enzyme solution of bacillus sp.SD771 obtained in the example 1 is added about 3%. After grinding this raw material, it mixes enough by the mixer, and it is a conventional method at a pellet mill (70 to 100 degree C internal temperature). Heating molding of the pellet was carried out, ventilation dryness was carried out at 70 degrees C, and the feed for fishery animals was produced. The underwater stability of this feed was also good. When the enzyme activity of an amylase was measured, 95% remained to additive enzyme activity. In addition, after making the activity of an amylase react at 50 degrees C among the 0.05MCHES buffer solution (pH 9.0), using fusibility starch as a substrate, it was made to color with an iodine-potassium iodide solution, and was determined from reduction in an absorbance.
[0029] 35% of [example 5] fish meals, and cornmeal 30%, 3% of MAKKARAMU salts, and vitamin premix 1.4%, the soybean meal was added to 0.01% of L-ascorbic-acid-2-magnesium phosphate, it considered as 100%, and the enzyme solution of bacillus sp.SD771 obtained in the example 1 was added about 3%, and after grinding this raw material and mixing enough by the mixer, it cast by the extruder. This molding was dried at the temperature of 100 degrees C with the dryer, and the expansion pellet feed for fishery animals was manufactured. When the enzyme activity of an amylase was measured, 82% of the addition remained to additive enzyme activity.
[0030] After adding the enzyme solution of bacillus sp.SD902 obtained in the example 2 after being referred to as a total of 100 at vitamin mix 0.04% amino acid mix 0.05% HEIKYUBU 8% 19% of barley, and beat pulp 18% [example 6] zea about 22% 14% [of soybeans], 6% [of soybean cake], 6% [of cottonseed meals], and cone gluten 6% about 3% and mixing enough by the mixer after trituration, the pellet was cast by the pelleter. This molding was dried at the temperature of 80 degrees C with the dryer, and the pellet feed for land animals was manufactured. When the enzyme

activity of xylanase was measured, 93% remained to additive enzyme activity. In addition, after making the enzyme activity of xylanase react by 50 degrees C and pH 7.0, using the birch xylan (hippo origin xylan) in 0.1M phosphate buffer solution (pH 7.0) as a substrate, it measured and determined the generated reducing sugar by 3 and 5-dinitro salicylic acid (DNS) which is a well-known method.

[0031] After adding the enzyme solution of bacillus sp.SD902 obtained in the example 2 after considering as a total of 100% at 0.3% [of salt], and vitamin premix 0.3% calcium 5% 10% of wheat, and grain sorghum 15% [example 7] zea about 35% 8% of fish meals, 8% of soybean cake, 15% of wheat bran, and alfalfa meal 3% about 3% and mixing enough by the mixer after trituration, the pellet was cast by the pelleter. This molding was dried at the temperature of 80 degrees C with the dryer, and the pellet feed for land animals was manufactured. When the enzyme activity of xylanase was measured, 89% remained to additive enzyme activity.

[0032] It ground, after adding the enzymes shown in the [example 8] table 1 in the feed of the respectively same composition as an example 6 and casting by the same manufacture method, and the survival rate of the enzymes in the feed immediately after manufacture was measured. Next, it was left for two weeks at the room temperature, and the survival rate of the enzymes in feed was measured similarly. In addition, the manufacture of the enzyme solution of NKS-21 is as follows, and its same is said of the case of the variant (S12->A) of NKS-21.

[0033] 2.0% soybean cake, 3.5% maltose, and 0.5% phosphoric-acid hydrogen 2 potassium, 0.05% magnesium sulfate, and liquid-medium 2L that consists of a sodium carbonate 1.0% were put into the jar fermenter of 5L **, and it sterilized for 20 minutes at 121 degrees C. The biomass of bacillus sp.NKS-21 cultivated by the same culture medium as this was inoculated, and the aeration spinner culture was aerobically performed at 35 degrees C for 48 hours. After cultivation, centrifugal separation of the culture medium was carried out by 6000rpm, and the biomass was removed. Next, the supernatant obtained by the above-mentioned method was condensed by ultrafiltration, and 200ml of enzyme solutions was obtained. Moreover, it is as [method / activity-measurement / NKS-21 and / of the variant (S12->A)] follows. Enzyme activity is a substrate. 0. Use Milk Casein in 1M Sodium-Carbonate-Borate-ized Potassium Buffer Solution (PH 10.0). After making it react by 30 degrees C and pH 10.0, a reaction is stopped with the 0.2M acetic-acid-sodium acetate solution containing 0.1M trichloroacetic acid. After filtering, the filtrate was made to color by the phenol reagent and it determined from the absorbance (an enzyme unit makes one katal the amount of enzymes which separates the trichloroacetic acid fusibility matter in which coloring in 660nm of the one mol considerable amount of thyrosins is shown in 1 second from the casein which is a substrate at 30 degrees C pH 10.0).

[0034]

[Table 1]

[illegible]の名称	SD902	SD771	NKS-21 変異体 (S12->A)	NKS-21
[illegible]	93%	82%	80%	35%
2[illegible]	88%	71%	64%	29%

[0035] Also not only in immediately after feed manufacture but in room temperature preservation, the high enzyme activity survival rate was obtained by using a thermostable enzyme (SD902, SD771, NKS-21 variant). At a non-thermostable enzyme (NKS-21), an enzyme activity survival rate is a low.

[0036] In order to check the effect over the feed of the enzyme of a [example 9] this invention, it experimented to straw and the straw trituration article, and it checked that effective activity existed. After decision or the mixer ground straw with a dry weight of 500g to 5cm length, it was under 33l. of water, and was left at the room temperature one whole day and night. Then, after having taken out straw, rinsing well and removing affixes, such as an effluent and mud, centrifugal hydroextraction of this was carried out, water was added so that a raw material might next become 15%, and it adjusted

to pH 7.0 and 70 degrees C. It added calmly and the enzyme solution of bacillus sp.SD902 obtained in the example 2 to this was agitated for 24 hours so that it might become 0.2% (pair kg dryness straw). After the reaction, after grinding and rinsing straw, centrifugal hydroextraction of this was carried out. When the lignin content of this was measured, it was changing to the straw (or trituration article) with which it was decreasing 25% and the slaking property has been improved 18% respectively. That is, this enzyme solution had the useful property which leads to a slaking-property improvement at least as an enzyme for feed. Moreover, the result in which it experimented similarly about the various enzyme solutions obtained in the example 2 and the example 3 is shown in Table 2. About what the effect was accepted in, O (especially a remarkable thing is O) and the thing which was not accepted filled in x. As a result, the useful effect of a slaking-property improvement was checked at least by the enzymes of this invention.

[0037]

[Table 2]

酵素溶液の種類	稲藁	稲藁粉砕品
SD401	○	○
SD402	○	◎
SD403	○	◎
SD902	◎	◎
SD401+NKS-21変異体(S12→A)	○	◎
SD403+SD516	◎	◎
SD403+SD771	◎	◎
SD403+SD772	◎	◎
NKS-21	×	×

[0038]

[Effect of the Invention] By this invention, even if it passes through processing by the heated type device, enzyme activity is fully held, and the constituent for feed which improved as the result is offered. The constituent for feed effective in using especially for the pellet is offered. Furthermore, the technology of this invention can be applied besides feed, for example, can use food, a medicine, etc. for an animal oral object (pass the mouth of an animal including a man, fishes, birds, etc. object taken in) widely. In the case of feed, the usage can apply.

[Translation done.]